Genetic Influences on Smoking: Candidate Genes

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Twin studies consistently indicate important genetic influences on multiple aspects of smoking behavior, including both initiation and cessation; however, knowledge regarding the role of specific genes is extremely limited. Habit-forming actions of nicotine appear to be triggered primarily at nicotinic receptors on the cell bodies of dopaminergic neurons in the mesolimbic "reward" system of the brain, a region implicated in addiction to other substances including cocaine, opiates, and alcohol. Important aspects of the dopaminergic pathway include synthesis of dopamine in dopaminergic neurons, release of dopamine by presynaptic neurons, receptor activation of postsynaptic neurons, dopamine re-uptake by presynaptic neurons, and metabolism of released dopamine. Research examining the role of allelic variation in genes involved in these functions is being actively pursued with respect to addictive behavior as well as personality traits and psycho- and neuropathologic conditions and has implications for smoking research. In addition, genetic differences in nicotinic receptors or nicotine metabolism might reasonably be hypothesized to play a role in smoking addiction. A role of dopaminergic or other genes in smoking cessation is of particular potential importance, as research in this area may lead to the identification of subgroups of individuals for whom pharmacologic cessation aids may be most effective. *Key words*: addiction, dopamine, genetic factors, nicotine, smoking. *Environ Health Perspect* 106:231–238(1998). [Online 24 March 1998]

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The reduction of adult use of tobacco has slowed considerably in recent years (*1*), highlighting the need for new insights into determinants of smoking behavior. It has been hypothesized that, as overall rates of smoking decline in response to social pressures, genetic influences may be particularly strong within the remaining population of smokers (*2*). Twin studies consistently indicate important genetic influences on multiple aspects of smoking behavior, including initiation, persistence, and cessation (*3–7*); however, knowledge regarding specific genes involved is extremely limited.

Recent evidence suggests that the addictive effects of tobacco constituents such as nicotine operate primarily through dopamine neurotransmission in the mesolimbic "reward" system of the brain, in a manner similar to that of other addictive substances such as cocaine, opiates, and alcohol (*8–11*). In May 1997, the U.S. Food and Drug Administration approved Zyban (bupropion) as the first non–nicotine-based smoking cessation aid; the mechanisms of action of this agent, while incompletely understood, involve dopaminergic stimulation (*12–14*). A role of dopaminergic genes in smoking cessation is thus of particular importance, as research in this area may eventually lead to the identification of genetic subgroups of individuals for whom dopaminergic cessation aids may be particularly efficacious.

Some candidate genes involved in dopaminergic function have previously been identified (*15,16*) and are of interest to researchers involved in a wide range of addictive and other behavioral disorders. These are genes that code for enzymes involved in the synthesis or metabolism of dopamine, dopamine receptors, and the dopamine transporter. For many of the genes of interest, polymorphisms have been only recently identified, and, among identified polymorphisms, there is a range of likely functional significance. Knowledge in this area is rapidly proliferating. Other genes, including some of the cytochrome P450 family as well as the nicotinic acetylcholine receptor, are involved in neural activity or metabolism of nicotine and have known polymorphisms, such that these genes may also be considered reasonable candidates to influence smoking behavior.

Evidence for Genetic Influences on Smoking Behavior: Twin Studies

Initial support for a genetic influence on the use of tobacco came from cross-sectional studies in twins and showed a mean heritability (that is, the proportion of phenotypic variation attributed to genetic variation) of cigarette smoking of 0.53 (range, 0.28–0.84) (*3,4*). Carmelli et al. (*4*) used data from a registry of male twins to examine hereditary influences on specific aspects of smoking behavior such as never smoking, former smoking, and quitting. A genetic influence on each of these aspects of tobacco dependence was observed. In an initial survey in 1967–1969, concordance was higher among mono- than dizygotic twins for both never and former smoking. Quitting during the subsequent 16-year interval was also more commonly observed among mono- than among dizygotic twins.

Heath and Martin (*5*) examined genetic influence on smoking persistence in a survey of Australian twin pairs and reported higher monozygotic correlations of smoking persistence in both sexes than the corresponding like-sex dizygotic correlations, consistent with the presence of a genetic influence. The genetic effect on persistence appeared to be unrelated to effects on smoking initiation. In a study of 434 female twin pairs in the Kaiser Permanente Women Twins Study, Edwards et al. (*7*) reported findings consistent with genetic influences on both former and current smoking at both the initial and 10-year follow-up examinations [concordance ratio in mono- versus dizygotic twins = 1.6; 95% confidence interval (CI) 1.0–2.3, and 1.8, CI 1.3–2.4, respectively]. This increased concordance in monozygotic twins remained apparent after adjustment for age, education, and frequency of contact between co-twins (a marker of the degree of similarity of environmental factors).

Shared genetic influences on psychopathology and smoking behavior, or on smoking and other addictive behaviors such as alcoholism, have been hypothesized. Kendler et al. (*17*), in a study of 1,566 female twins, reported an association between smoking and major depression that appeared to reflect a shared genetic predisposition to both conditions. Swan et al. (*18*), using data from the National Heart, Lung, and Blood Institute's Twin Study, reported a common genetic influence on smoking, alcohol and coffee use, with 36% of the heritability of smoking attributed to a shared susceptibility to these substances.

Although twin studies provide supportive evidence for the role of genes in smoking behavior, none have provided clues as to specific genes that may be involved. Further, the interpretation of twin studies is limited by their assumption that environmental influences are similar for monozygotic and dizygotic twins; thus, if monozygotic twins interact with and influence one another more than do dizygotic twins, estimates of heritability may be inflated (*19*). Studies of specific

candidate genes will be important to advance knowledge of genetic influences on smoking.

The Dopaminergic Reward System and Addictive Behavior

Addictive drugs stimulate a neural "reward pathway" in the the limbic system of the brain, which controls basic emotions and behaviors and provides a physiological basis for the perception of pleasure (20). Pathways of this system, which originate in the ventral tegmental area (VTA) and travel primarily to the nucleus accumbens (NA), are composed of dopaminergic neurons. Amphetamine and cocaine increase extracellular dopamine by displacing it from presynaptic sites and by blocking dopamine re-uptake, respectively. Opiates, ethanol, and nicotine increase extracellular dopamine by stimulating the firing of dopaminergic neurons (21). In addition, addiction to these substances may alter the normal production of dopamine, so that discontinuing use after addiction has occurred may trigger withdrawal (9,20,22).

Appreciation of the importance of the dopamine pathway in addictive behavior has led to increasing interest in the design of therapies aimed at this pathway (23,24). Also, research examining the role of allelic variation in genes involved in dopamine synthesis, transport, receptor activity, and degradation is being actively pursued with respect to addictive behavior (16,25,26), personality traits (27,28), and psycho- and neuropathologic conditions (15,25).

Effects of Cigarette Smoking and Nicotine on the Dopaminergic Reward System

Nicotine is believed to be the primary component of tobacco that motivates continued use (29). The behavioral and neurobiological effects of nicotine are similar to other drugs that are known to be addictive (8,30). Habit-forming actions of nicotine appear to be triggered primarily at nicotinic receptors on the cell bodies of dopaminergic neurons in the VTA that project to the NA—i.e., the "reward" pathway (9-11). In animal studies, nicotine stimulates dopamine neurotransmission in the outer shell of the NA in a manner similar to that of other addictive drugs such as cocaine, amphetamine, and morphine (8). Nicotine-induced dopamine release is significantly reduced by mecamylamine, an antagonist of central nervous system (CNS) nicotinic receptors, but is not influenced by blockage of peripheral (non-CNS) nicotinic receptors (21). Destruction of the mesolimbic system attenuates the rewarding effects of intravenous nicotine (31). VTA injections of the nicotinic agonist cytisine are rewarding, and both VTA and systemic injection of

dopamine antagonists disrupt intravenous nicotine self-administration (9,11).

Experimental evidence in humans also supports a role for dopaminergic processes in nicotine addiction, although the precise response to nicotine antagonists differs. In both human cigarette smokers and animals exposed to intravenous nicotine self-administration, use of nicotine decreases over time in the presence of the nicotinic antagonist mecamylamine. Unlike animals, however, human smokers show an initial transient increase in smoking, which has been hypothesized to represent an attempt to compensate for nicotinic receptor blockade (11).

Diverse neurologic disease processes provide further indirect evidence of an interrelationship between cigarette smoking and dopaminergic effects. Schizophrenia is associated with alterations in dopaminergic transmission, and the effect of nicotine on midbrain dopamine systems has been suggested to partially explain the extremely high prevalence (90%) of tobacco smoking in schizophrenics (10,32). In contrast, a negative association of smoking with risk of Parkinson's disease, a condition characterized by loss of nigrostriatal dopamine neurons, has been consistently reported (33,34). The efficacy of nicotine as a treatment for tremor in Parkinson patients is currently under investigation (35). It has also been suggested that the mesolimbic dopamine system is involved in some forms of depression (10).

Compounds other than nicotine in tobacco may also affect the rewarding effects of cigarette smoking. Monoamine oxidases A and B, which are involved in the oxidation and degradation of dopamine, are partially inhibited in the brains of smokers due to a tobacco constituent other than nicotine (36,37). Increased availability of dopamine to chronic smokers arising from this inhibition may enhance the addictive power of nicotine. Also, neurologic pathways other than the mesolimbic pathway may be directly or indirectly affected by tobacco constituents and may contribute to other reinforcing effects and learned behaviors that play a role in smoking addiction (29).

Candidate Genes for Smoking Behavior: Dopaminergic System

Important components of the dopaminergic pathway include 1) synthesis of dopamine

in dopaminergic neurons; 2) release of dopamine by presynaptic neurons; 3) receptor activation of postsynaptic neurons; 4) dopamine re-uptake by the presynaptic neuron, and 5) metabolism of released dopamine. Many candidate genes involved in dopaminergic neurotransmission have been characterized and have identified polymorphisms, including those for tyrosine hydroxylase (TH), the dopamine transporter (DAT1), dopamine receptors (DRD1–DRD5), dopamine β-hydroxylase (DBH), catechol-o-methyltransferase (COMT), and monoamine oxidase (MAO) A and B (Table 1) (15).

In addition to smoking, genes involved in dopamine function are of interest to researchers in a variety of areas, including other addictive behaviors (such as alcoholism and cocaine abuse), as well as disorders that occur more or less commonly among smokers or substance abusers than in the general population (such as schizophrenia, affective disorders, and Parkinson's disease). I have drawn from this broad literature, in addition to genetic studies specific to smoking, in the following discussion of specific polymorphisms of dopaminergic genes that may play a role in smoking behavior.

Synthesis

Tyrosine, an amino acid that is abundant in dietary proteins, is the starting point for biosynthesis of dopamine. Tyrosine enters dopaminergic neurons and is converted to dihydroxyphenylalanine (L-DOPA) by TH; this reaction is considered the rate-limiting step in dopamine biosynthesis (38,39). Subsequent conversion of L-DOPA to dopamine involves the action of an additional enzyme, aromatic amino acid decarboxylase.

The TH gene has been evaluated in many disorders related to dopaminergic function, including alcoholism, bipolar affective disorder, unipolar affective disorder, schizophrenia, and Parkinson's disease (40–46). Initial studies made use of biallelic restriction fragment length polymorphisms (RFLPs), including a TaqI site 1 kb upstream of the TH transcription initiation site, a BglII RFLP located at the 3' end of the TH gene, and a PstI RFLP (47).

A five-allele tetranucleotide repeat polymorphism in the first intron of the TH gene has also been identified (48) and has

Table 1. Role of identified genes involved in the dopaminergic reward pathway

Function	Relevant genes
Dopamine synthesis	Tyrosine hydroxylase (TH)
Receptor activation	Dopamine receptors (DRD1, DRD2, DRD3, DRD4, DRD5)
Re-uptake	Dopamine transporter (DAT1)
Metabolism	Catechol-o-methyltransferase (COMT)
	Monoamine oxidase A and B (MAO A, MAO B)
	Dopamine β-hydroxylase (DBH)

been used in a number of studies (40,42,44,46,49,50). Five different DNA fragments (260, 256, 252, 248, and 244 bp) were denoted as K1, K2, K3, K4, and K5 alleles, respectively, according to Polymeropoulos et al. (48). Using this polymorphism, Jonsson et al. (39) reported a correlation of TH genotype with homovanillic acid (HVA) concentrations (a measure of brain dopamine metabolism) in the cerebrospinal fluid of healthy individuals. Individuals with at least one copy of the K1 allele (69.7%) had significantly reduced HVA levels relative to individuals lacking the K1 allele (30.3%; mean HVA = 165 vs. 206 nmol/l, respectively; p = 0.032). It has been suggested that first-intron sequences in the TH gene may play a role in alternative splicing events that result in four types of mRNA of this gene (40).

Most recently, a base pair mutation leading to a Val-81 to Met substitution in the second exon of the gene has been reported (51) and used in an association study of bipolar disorder (52). The Val-81 to Met polymorphism was present in heterozygous form in 57.1%, and in homozygous form in 11.5%, of healthy individuals in a German study (51).

Re-uptake

In presynaptic dopaminergic neurons, dopamine is transported from the cytoplasm to specialized storage vesicles. With the arrival of an action potential, these vesicles discharge their contents into the synapse (38,53). Presynaptic nerve terminals possess specialized dopamine transporters. Termination of neurotransmission is accomplished by rapid removal of dopamine from the synapse by re-uptake into the nerve terminal from which it was released via the dopamine transporter or by being metabolized into an inactive compound (32).

A polymorphic 40-base variable number tandem repeat (VNTR) has been identified in the 3′ untranslated region of the DAT1 gene (54). Although it is unlikely this VNTR has functional relevance, it is close to the coding region and may be in linkage disequilibrium with a mutation that alters gene expression or protein structure; to date, no functional mutations of DAT1 have been reported (55). In white and black Americans, DAT1 VNTR copy number varies between 3 and 11, with more than 90% of individuals displaying alleles with 9 or 10 copies (54,56). Associations of the DAT1 VNTR with attention-deficit disorder, schizophrenia, cocaine-induced paranoia, and alcoholism accompanied by delirium or withdrawal seizures have been reported (55–58). Association studies examining the role of DAT1 in other disorders have been negative (49,59). In 1997, Caporaso et

al. (60) reported a significant association between DAT1 genotype (specifically, with presence of the 9-repeat allele) and smoking in a comparison of 233 smokers and 233 race- and gender-matched controls.

Receptors

Multiple types of dopamine receptors have been identified and characterized and can be grouped into two classes (38,61–64). The D1 class of dopamine receptors contains the D1 and D5 (sometimes referred to as D1A and D1B, respectively) receptor subtypes; the D2 class includes the D2, D3, and D4 receptor subtypes. When activated, D1-like subtypes stimulate adenylate cyclase activity and increase cyclic AMP; D2-like receptor subtypes generally inhibit adenylate cyclase activity. Differences among receptor subtypes in their response to various pharmacologic agents is currently under investigation in the development of new therapies for conditions such as Parkinson's disease, schizophrenia, and addictive disorders (38,61–65). Polymorphisms of each of the dopamine receptor genes (DRD1, DRD2, DRD3, DRD4, and DRD5) have been identified and examined in association studies of various conditions influenced by dopaminergic neurotransmission.

DRD1. Four polymorphic sites of DRD1 were initially identified by single-strand conformational analysis (SSCA) (66). These include two sites in the 5′ untranslated region (UTR); one rare, silent polymorphism in codon 421; and one site in the 3′ UTR. Mutation screening of the coding sequence of this gene in 131 schizophrenics using dideoxy fingerprinting identified no polymorphisms that altered the amino acid sequence (67). The 5′ flanking region of DRD1 contains a functional promoter; screening for mutations in this region among individuals with schizophrenia, bipolar disease, and healthy controls using SSCA revealed six single base substitutions, none of which was located in regions known to have an influence on transcriptional activity (68). Using the Bsp 1286I RFLP of DRD1 located in the 3′ UTR, no evidence was found for an association with alcoholism (69). In a study of smoking and DRD1 polymorphisms that used the DdeI polymorphism located in the 5′ UTR, a significant increase in the frequency of homozygosity for either the DRD1 1- or 2-allele was reported in smokers relative to nonsmoking controls (70). Among smokers, homozygosity of DRD1 was also associated with number of cigarettes smoked per day. The mechanism of action of this apparent heterozygous advantage/disadvantage (if real) is unknown. These authors suggest that, because the DRD1 DdeI polymorphism is a neutral base change,

the association, if real, may reflect linkage disequilibrium with mutations in regions at or near the DRD1 locus that affect receptor function or density.

DRD2. Following an initial report of a positive association between alcoholism and presence of the A1 allele of a TaqI RFLP (located 10 kb downstream of the coding sequence of DRD2), DRD2 has been the focus of numerous association studies examining alcoholism, cocaine addiction, smoking, schizophrenia, and other disorders. A search for functional variants in the coding region (exons 2–8) of DRD2 identified three infrequent variants that altered the amino acid sequence of the receptor (Ser-311 to Cys, Val-96 to Ala, and Pro-310 to Ser) (71,72). In 1997, a search for functional variants in the promoter region (5′ flanking region and first exon) of the gene was conducted; two polymorphisms were identified, and cell culture experiments suggest reduced function associated with the deletion allele of one of these polymorphisms (-141C Ins/Del) (73). Association studies of DRD2 have not yielded consistent results and have generated considerable controversy, particularly regarding the possible role of DRD2 in alcoholism (74–78). While the bulk of research published to date has used the Taq1 allele system, other nonfunctional RFLPs have also been examined, as has the Ser-311 to Cys polymorphism. The recently identified -141C Ins/Del polymorphism has been assessed in only one study to date, in which a reduced frequency of the -141C Del allele was reported among 260 schizophrenic patients relative to 312 controls [odds ratio (OR) = 0.6, CI 0.44–0.81) (73).

Recent contributions to the DRD2 literature have noted the need for additional studies of DRD2 and substance abuse and the need for improved methodology. Two strategies have been recommended, including further examination of functional variants of DRD2 (79) and DRD2 haplotypes, as a means to reduce the possible confounding effects of ethnic variation in DRD2 allele prevalence (74,80,81). Some evidence suggests that the Taq1 A polymorphism is in linkage disequilibrium with a functional allelic determinant, including reported correlations with DRD2 receptor number or density (78,82) and with brain regional glucose metabolism (83). Also, a better response to treatment with a dopamine receptor agonist has been reported among alcoholics who carry the A1 allele than among those who do not (24,84).

A few studies have assessed DRD2 polymorphisms and smoking behavior. The Taq1 A1 allele was initially noted to be more prevalent in smokers or ex-smokers relative to nonsmokers (OR = 2.15 and

1.71, respectively) (85). In a subsequent study, the A1 allele was most common among those smokers who reported the least success in quitting smoking, i.e., among those whose maximum quit attempt was 1 week or less, 57.4% had at least one copy of the A1 allele, while among those who had quit for 6 months or more, 33.3% had the A1 allele. The prevalence of the A1 allele also declined as the age at which subjects first started smoking increased, and was higher among those who smoked greater amounts per day (86). In a third study (60), among a subgroup of individuals with the DAT1 9-repeat allele, there was a significant association of smoking status with the DRD2 A1 allele, which was not evident in the study population as a whole.

DRD3. Expression of DRD3 appears restricted to areas of the brain, including the NA, that are thought to play a significant role in addictive behavior (87). Lannfelt et al. (88) reported a common point mutation (A to G) in the first exon of DRD3, resulting in an amino-acid substitution from serine to glycine at codon 9 (Ser-9 to Gly), located at the N-terminal extracellular domain of the receptor. This amino acid substitution may modify incorporation of the receptor protein into the membrane (89). A number of studies have assessed the relation of the DRD3 Ser-9 to Gly polymorphism with schizophrenia [summarized in Ebstein et al. (90)]. Several have reported excess homozygosity (of either allele) of the Ser-9 to Gly polymorphism among schizophrenic patients; others (90,91) have observed an increased prevalence of allele 2 (coding for glycine) in schizophrenics or no association. Multiple studies have also been conducted to assess the role of the Ser-9 to Gly polymorphism in alcoholism (69,92–94) and cocaine dependence (95). Although one study (69) reported an increased frequency of DRD3 allele 1 among alcohol-addicted patients with delirium (p = 0.04), most studies have found no association.

DRD4. The third exon of DRD4 contains an unusually polymorphic 16-amino acid repeat, and physiologic differences in ligand binding have been observed between the short (2–5 repeats; most commonly 4) and long (6–10 repeats; most commonly 7) forms of this repeat. In 1996, two studies conducted in different populations (one in Israel and one in the United States) reported an association between the long allele of the DRD4 VNTR and the normal personality trait of "novelty seeking" (27,28). A subsequent study conducted in Finland (96) failed to confirm this association. Disease-related research involving this polymorphism has included studies of Parkinson's disease, bipolar disorder, schizophrenia, and alcoholism, most of which

have not reported associations of the exon 3 DRD4 polymorphism and disease (97). Other expressed polymorphisms in DRD4 are also known to occur, some of which have been examined in association studies, including a 12 bp duplication polymorphism and a 13 bp deletion, both located in exon 1 (97–100).

DRD5. To date, the DRD5 gene has been examined in only a few linkage studies of bipolar disorder, schizophrenia, or Tourette's syndrome, with no evidence for a major effect of this gene reported. A common, silent polymorphism (Pro-326) has been reported (101). Five polymorphisms coding for amino acid changes have recently been identified for the D5 receptor; one of these, a rare nonsense change predicted to result in a 50% diminution of functional protein, has been examined in a case–control study of schizophrenia (102). A polymorphic dinucleotide repeat in the DRD5 promoter has also been identified, but is not believed to be of functional significance (103).

Metabolism

The major end product of dopamine metabolism in primates is HVA. Enzymes responsible for metabolism of dopamine to HVA are COMT, MAO A and B, and aldehyde dehydrogenase (38). An alternative metabolic pathway for dopamine leads to the formation of norepinephrine; the DBH enzyme catalyzes this conversion (104).

COMT. COMT is considered a key regulator of dopaminergic neurotransmission (38,105). A common biallelic functional polymorphism occurs within the COMT gene resulting in high- and low-activity gene products and a three- to fourfold variation of enzyme activity. This is due to a G to A transition in the fourth exon of the gene, resulting in a valine to methionine substitution at codon 158 of the membrane-bound forms of COMT (corresponding to codon 108 of the soluble or cytoplasmic form) (106). Homozygosity for the allele encoding methionine is associated with low enzyme activity and thermolability; homozygosity for the allele encoding valine is associated with high activity and thermostability; and heterozygous individuals have an intermediate level of COMT activity and heat stability (106). To date, this COMT gene polymorphism has been examined in relation to Parkinson's disease, schizophrenia, obsessive compulsive disorder, and bipolar disorder (105,107–115), with most studies showing no association. An intriguing possible association with substance abuse has been noted, with a higher proportion of substance abusers than drug-free controls having high activity COMT genotypes (25).

MAO A and B. Genes encoding MAO A and B are closely linked and are located on the X chromosome. Abnormal MAO enzyme activity levels have been associated with a variety of conditions, including smoking, alcoholism, schizophrenia, and Parkinson's disease (36,37,116,117). In 1996, Fowler et al. reported that the brains of individuals who currently smoke show a 40% reduction in MAO B enzyme relative to never or former smokers (36), and that MAO A enzyme levels are lower in smokers than nonsmokers (37). In a cessation trial, heavy smokers treated for 3 months with moclobemide, a reversible inhibitor of MAO A enzyme, had superior abstinence rates for up to 6 months after quitting compared with heavy smokers treated with placebo (118).

Available data suggest that MAO enzyme activity (measured in platelets and fibroblasts) is under genetic control of a single major locus, possibly the MAO locus itself, through noncoding, regulatory elements (117,119). Several polymorphisms of the MAO-A and MAO-B genes have been identified; three polymorphisms of the MAO-A gene have been shown to be associated with enzyme activity level, although they do not affect the amino acid sequence (119). One of these, located in exon 14 of the MAO-A gene, has been assessed in a case–control study of Parkinson's disease, in which no assocation with disease risk was evident (117).

Recently, the coding region of the MAO-B gene was screened for mutations by dideoxy fingerprinting in 100 schizophrenic patients; none were identified among the 80 white patients included in that study (116). A single base difference (A or G) in intron 13 of the MAO-B gene has been assessed in case–control association studies of Parkinson's disease and of schizophrenia. In some studies, the A allele was associated with an approximately twofold increased risk of Parkinson's disease (120,121), while in another study (122), elevated risk of this disease was observed among individuals with the G allele. The effect of this polymorphism on brain activity, if any, is unknown; however, Costa et al. (122) note that it is located in close proximity to the 3′ splicing acceptor site of intron 13 and could conceivably be involved in mRNA processing. No association of this polymorphism with risk of schizophrenia has been observed (117).

DBH. DBH catalyzes the conversion of dopamine to norepinephrine. The DBH gene has been examined in linkage and association studies of schizophrenia (123,124) and bipolar disorder (125). High serum DBH enzyme activity has been associated with alcohol consumption (126);

also, an association of serum DBH activity with an intron 9 (two-allele) polymorphism of this gene has been reported (*127*). A six-allele polymorphic $(GT)_n$ repeat has also been identified, with two alleles accounting for more than 90% of alleles present in individuals. It appears that DBH activity is associated with this polymorphism (*124,127*), with individuals homozygous for the A3 and A4 alleles having lowest and highest DBH activity, respectively, and A3/A4 heterozygotes in an intermediate range.

Other Interacting Neurotransmitters

Dopaminergic neurotransmission is influenced by other neurotransmitters, such as serotonin and norepinephrine (*39,128*). Interactions between dopamine and other neurotransmitter systems have been hypothesized to potentially influence risk of some neurologic conditions (e.g., schizophrenia, depression), as well as response to therapy. Polymorphisms of a variety of genes involved in serotonergic neurotransmission have been identified and, in a few cases, examined in association studies (*15*). A recent study (*129*) reported an association between the personality trait of reward dependence and a cysteine-to-serine substitution in a serotonin receptor (HTR2C); there was also a significant interaction between polymorphisms of the dopamine receptors DRD3 and DRD4 and the serotonin polymorphism on reward dependence.

Other Candidate Genes for Smoking Behavior: Activity and Metabolism of Nicotine

Nicotine is considered the most addictive component in tobacco products (*130*). Nicotine, via binding to neuronal nicotinic receptors, enhances dopamine release and neurotransmission in the mesolimbic dopamine system. Chronic nicotine use results in an increased density of nicotinic receptors in various brain areas, and it is thought that this increase may somehow influence dependence on nicotine. One model that has been proposed is that chronic exposure to nicotine induces inactivation of some receptors, which then turn over more slowly, resulting in an increased number of nicotinic receptors present. It has been suggested that nicotine-induced release of dopamine drives tobacco usage, while receptor inactivation due to chronic nicotine use may play a role in the processes of tolerance and withdrawal (*29*).

Genetic differences in nicotinic receptor activity or nicotine metabolism might reasonably be hypothesized to play a role in smoking addiction. Nicotinic cholinergic receptors are diverse in subunit structure,

function, and distribution within the nervous system; this diversity is likely to mediate the complex actions of nicotine in tobacco users (*131*). Nicotinic receptors are present in the brain, autonomic ganglia, and the neuromuscular junction. Neuronal nicotinic acetylcholine receptors are believed to be most relevant to nicotine addiction; these are located throughout the brain and are composed of α and β subunits, most commonly (90%) as the α_4, β_2 receptor (*131*). The identification and characterization of polymorphisms in the α_4 subunit of the neuronal nicotinic acetylcholine receptor (CHRNA4) is an area of active research (*132–135*). This gene has been hypothesized to play a role in various neurologic conditions including epilepsy and panic disorder. To date, only one association study has been conducted; in that study, no differences in the allele frequency of any of three silent polymorphisms of the CHRNA4 gene were noted in a comparison of 88 patients with panic disorder and healthy controls (*133*).

Considerable interindividual variability exists in the metabolism of nicotine (*131*). Approximately 70–80% of nicotine is metabolized to cotinine in a two-step process. The first step, in which nicotine is converted to nicotine iminium, is metabolized by a CYP450 enzyme, most likely CYP2A6 (*131,136*). Interindividual differences in the activity of CYP2A6 exist, and two variant *CYP2A6* alleles (*CYP2A6v1* and *CYP2A6v2*) have been identified (*137,138*). *CYP2A6v1* has a T to A mutation, creating an amino acid change (Leu-160 to His), and *CYP2A6v2* has a C to A mutation; both mutations occur in exon 3. Subjects homozygous for *CYP2A6v1* have undetectable coumarin metabolism (a process dependent on CYP2A6), whereas heterozygotes for *CYP2A6v1* have levels of coumarin metabolism that are 50–60% that of the general population. The functionality of CYP2A6v2 is not yet known.

Another P450 enzyme, CYP2D6, is known to be polymorphic, and in white populations is inactive in 5–10% of individuals (*139*). Individuals are often referred to as poor metabolizers (PMs) or extensive metabolizers (EMs), based on a correlation of *CYP2D6* genotype with the phenotypic ability to metabolize debrisoquine; PMs lack CYP2D6 activity because of inactivating mutations in both alleles of the gene (located at 22q13.1). CYP2D6 has been suggested to be involved in the metabolism of nicotine (*140*), although this has recently been questioned (*141*). CYP2D6 is active in both the liver and the brain, and the distribution of CYP2D6 is similar to that of the dopamine transporter (*142*). Some research suggests that personality characteristics may differ by

CYP2D6 genotype and/or the associated debrisoquine metabolizer phenotype, and it has been hypothesized that an endogenous neuroactive substance involved in dopamine neurotransmission may be a substrate of CYP2D6 in brain (*142–144*). In some studies, CYP2D6 PMs have been observed to be at increased risk of Parkinson's disease, and studies are currently in progress to evaluate the role of CYP2D6 in opiate addiction (*145*). Turgeon (*146*) reported that none of 58 healthy smokers <35 years of age had the PM phenotype, compared to 10% of 200 nonsmoking white controls from the same population. In contrast, Cholerton et al. (*147*) found no difference in *CYP2D6* genotype in a comparison of 100 cigarette smokers with 104 nontobacco users aged 18–61.

Data regarding correlations between smoking prevalence and polymorphisms in other P450 enzymes are extremely limited. Recently, an association of the CYP1A1 *MspI* genotype with pack-years of smoking was reported in two independent samples of healthy, white control populations (*148*).

Summary

Twin and family studies have contributed evidence of a genetic component in smoking addiction and in specific aspects of smoking behavior such as initiation and quitting. These studies provide an impetus for the identification of specific genes that may be involved. A rather large number of candidate genes for smoking behavior—that is, genes involved in dopaminergic neurotransmission or in the activity and metabolism of nicotine—have been identified; however, few studies have examined the relation of these genes to smoking. For many of the genes of interest, polymorphisms have been identified only recently. Among identified polymorphisms, there is a range of likely functional significance, including those of known, possible, and unknown functionality. Given the rapidity with which knowledge regarding dopaminergic genes is accumulating, as well as the possibility that other neurologic gene products (e.g., those involved in serotonergic gene transmission) may also influence addictive behavior, it is likely that additional gene polymorphisms of relevance to smoking behavior will be identified during the next several years.

As population rates of smoking decline in response to social pressures, genetic influences may be particularly strong among the remaining smokers. Understanding the genetic influences on smoking initiation may allow targeting of prevention strategies to individuals at highest risk. Increased understanding of genetic influences on the ability to quit smoking may lead to the

identification of genetic subgroups of individuals who are most likely to benefit from particular pharmacologic interventions for smoking cessation.

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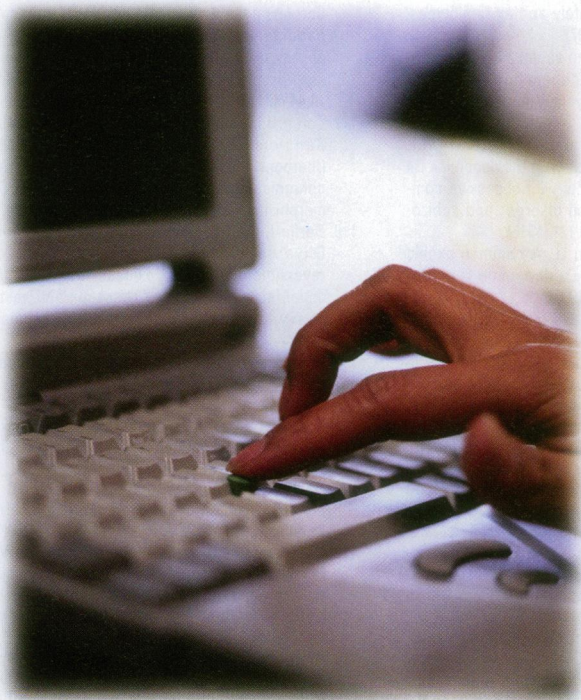
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